

The University of Chicago

ACTIVE AND PASSIVE IMMUNIZATION IN EXPERIMENTAL POLIOMYELITIS

A PART OF A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1936

By
FRANCIS BYRON GORDON

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FRANCIS BYRON GORDON

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ACTIVE IMMUNIZATION

A variety of virus preparations given by various routes has been used in attempts to produce an active immunity against experimental poliomyelitis. The results of these numerous researches¹ indicate that an immunological response may be detected in some monkeys after either untreated virus or certain types of treated virus are given by extraneural routes. In general it appears that relatively large amounts of virus-bearing tissue must be injected to induce an antibody response detectable by the serum neutralization test.

In the experiments reported here a study was made of the immunizing capacity of the complex formed by adsorption of poliomyelitis virus to alumina gel. A portion of this work has been presented elsewhere as preliminary reports² and the interpretation of the conclusions has been incorporated into a previous paper.³

The antigenicity of a number of different substances adsorbed to alumina gel has been studied. The following reports offer diversified examples. Plaut and Rudy⁴ induced antibody formation in rabbits by injection of an alcoholic extract of beef brain mixed with alumina gel, when the brain extract alone was not antigenic. Hektoen and Welker⁵ have reported the continued maintenance of a high precipitin level in rabbits following one injection of various proteins adsorbed to alumina gel. This agent has also been used for adsorption of diphtheria toxoid (Schmidt⁶), tetanus toxoid (Schmidt and Steenberg⁷) and scarlatina toxin (Farago⁸). Production of antibody by injection of a carbo-

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1. A review of this subject to 1932, may be found in "Poliomyelitis," International Committee for the Study of Infantile Paralysis, Baltimore, Williams and Wilkins Company, 1932. More recent reports are included below. Untreated virus: Brodie, M.: *Proc. Soc. Exper. Biol. & Med.* **30**: 1238, 1933; treated with formalin: Brodie, M.: *J. Immunol.* **28**: 1, 1935, Schultz, E. W., and Gebhardt, L. P.: *California and West. Med.* **43**: 111, 1935, Olitsky, P. K., and Cox, H. R.: *J. Exper. Med.* **63**: 109, 1936, Kramer, S. D.: *J. Immunol.* **31**: 167, 1936; treated with sodium ricinoleate: Kolmer, J. A.: *J. Immunol.* **32**: 341, 1937, Kramer, S. D., and Grossman, L. H.: *J. Immunol.* **31**: 183, 1936; virus-immune serum mixtures: Kramer, S. D., Schaeffer, M., and Park, W. H.: *J. Immunol.* **27**: 199, 1934, Kramer, S. D.: *J. Immunol.* **31**: 191, 1936; references 9 and 10.

2. Gordon, F. B., Harrison, Jas. A., and Hudson, N. P.: (a) *J. Bact.* **29**: 62, 1935; (b) *Proc. Soc. Exper. Biol. & Med.* **32**: 689, 1935.

3. Hudson, N. P., Lennette, E. H., and Gordon, F. B.: *J.A.M.A.* **106**: 2037, 1936.

4. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **81**: 87, 1933.

5. *J. Infect. Dis.* **55**: 271, 1933.

6. *Compt. rend. Soc. d. biol.* **106**: 765, 1931.

7. *Acta. path. et microbiol. Scandinav.* **13**: 401, 1936.

8. *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **92**: 220, 1938.

hydrate and alumina gel has been reported (Uhlenhuth and Remy⁹).

In the field of filtrable viruses immunization by means of virus adsorbed to a colloidal carrier has been attempted. In these experiments the purpose has been mainly to render the virus incapable of producing the disease, without destroying its capacity for immunization. Kodama¹⁰ used vaccine virus adsorbed to aluminum hydroxide and to ferric hydroxide for immunization of rabbits. Olitsky and Cox¹¹ succeeded in immunizing guinea pigs against equine encephalomyelitis by means of virus adsorbed to alumina gel. Schmidt and his coworkers have studied the adsorption of a number of viruses to alumina gel and have reported on the immunizing capacity of adsorbates of fowl plague¹² and of foot and mouth disease.¹³ The successful immunization of monkeys against poliomyelitis with virus adsorbed to the same agent has been reported by Rhoads.¹⁴ Since the completion of the present work Kramer, Grossman and Hoskwith¹⁵ have also reported success in immunizing monkeys with similar preparations.

In the work presented here monkeys were given subcutaneous injections of virus adsorbed to alumina gel while others received the eluate from such mixtures and others crude virus emulsion for purposes of comparison. The presence of an immunologic response was ascertained by the *in vitro* serum neutralization test and by intranasal test inoculation. The response to virus adsorbed to alumina gel was studied also in sheep.

MATERIALS AND METHODS

Rhesus monkeys (*Macaca mulatta*) of 1,300 to 3,500 gm. weight were used. Daily temperature records and observations were made, and at death portions of cervical and lumbar cord were taken routinely for histological examination.

Alumina gel was prepared according to the method described by Willstaetter¹⁶ for his aluminum hydroxide gel, type C. The details of his method were followed closely, except that sedimentation was accomplished by centrifugation instead of by gravity. Bits of pooled, glycerolated spinal cords of monkeys succumbing to inoculation with the MV strain of virus were ground with sand and made up to 6% emulsion in phosphate buffer of p_H 7.4. After light centrifugation, the supernatant was treated with ether, according to the method described by Clifton, Schultz and Gebhardt.¹⁷ The resulting water-clear solution was rid of traces of ether by boiling *in vacuo* for a short time, brought to an acid reaction of p_H 6.5 and shaken with an equal volume of alumina gel. After centrifugation one-half the total volume was removed as supernatant. The remainder, after being thoroughly shaken, constituted the complex which will be spoken of as "virus-gel." Such a preparation represented approximately twice the volume of the original cord emulsion. In some experiments a "nervous tissue-gel" was used as a control. This was prepared in the same way as virus-gel except that nervous tissue (brain and cord) of non-poliomyelitic monkeys was used. Elution of virus from such a virus-gel complex, originally

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9. Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**: 171, 1938.
 10. Kitasato Arch. Exper. Med. **10**: 243, 1933.
 11. J. Exper. Med., **63**: 311, 1936.
 12. Acta path. et microbiol. Scandinav. **12**: 262, 1935.
 13. Ztschr. f. Immunitätsforsch. u. exper. Therap. **92**: 392, 1938.
 14. J. Exper. Med. **53**: 399, 1931.
 15. J. Immunol. **31**: 199, 1936.
 16. Untersuchungen über Enzyme, Berlin, Julius Springer, 1928.
 17. J. Bact. **22**: 7, 1931.

described by Sabin,¹⁸ was accomplished by shaking with phosphate buffer at a reaction of pH 7.4. After the centrifugation the supernatant contained the eluted virus.

For intranasal inoculation as a test of immunity, the monkey was anesthetized, the nasal cavity washed out with an M/15 solution of KH_2PO_4 (Schultz and Gebhardt),¹⁹ and 1 to 2 cc. of 10% distilled water emulsion of virus was dropped into the nostrils.

Serum neutralization tests were set up as follows: Glycerolated virus cord was made up to a 10% emulsion in physiological saline and centrifuged (30 minutes at 1,500 R.P.M.), and the supernatant diluted with saline to make a 1% emulsion. One-half cc. quantities of this emulsion were added to tubes in which 1.5 cc. of the serums in dilutions to be tested had previously been placed. Positive and negative controls were always included. All tubes were allowed to remain in the incubator at 38 C. for 2 hours, and in the refrigerator over night. The entire contents of a tube were then inoculated intracerebrally into a monkey. The results are given in terms of original serum dilution and not the final dilution after virus was added.

EXPERIMENTS WITH MONKEYS

Experiment 1.—Six monkeys were injected subcutaneously with virus-gel, and 6 with the eluate from virus-gel. As controls 2 were given nervous tissue-gel. From 1 to 5 injections of 10 cc. each at 3-day intervals were given subcutaneously according to the schedule give in table 1. Each injection contained theoretically the amount of virus present in 0.3 gm. of the original tissue. One monkey developed poliomyelitis during the immunization. One month after the final immunizing injection the remaining monkeys were bled for a sample of serum and given intranasal inoculations of virus. Although the 11 serums from monkeys which had received virus possessed neutralizing ability, the monkeys were, with 2 exceptions, not resistant to the test inoculations by the nasal route.

TABLE 1.—Active Immunization with Varying Doses of Virus-Gel and Eluate

Monkey No.	Immunization (10 cc. Quantities Subcutaneously at 3-day Intervals)	Result of Intranasal Virus Inoculation	Result of Serum Neutralization Test
1	Virus-gel, 1 injection	No poliomyelitis	+
2	Virus-gel, 1 injection	Poliomyelitis	+
3	Virus-gel, 3 injections	Poliomyelitis	+
4	Virus-gel, 3 injections	Poliomyelitis	+
5	Virus-gel, 4* injections		
6	Virus-gel, 5** injections		+
7	Eluate, 1 injection	Poliomyelitis	+
8	Eluate, 1 injection	Poliomyelitis	+
9	Eluate, 3 injections	Poliomyelitis	+
10	Eluate, 3 injections	Poliomyelitis	+
11	Eluate, 5 injections	No poliomyelitis	+
12	Eluate, 5 injections	Poliomyelitis	+
13	Normal nervous tissue-gel, 5 injections (control)	Poliomyelitis	0
14	Same (control)	No poliomyelitis	0

+ = neutralization, 0 = no neutralization.

* Poliomyelitis occurred 2 days after fourth injection.

** Animal killed 30 days after last injection (exper. 5).

The 3 animals which resisted intranasal inoculation were later given virus intracerebrally. Monkey 11 resisted, the others succumbed.

The virus-gel and eluate preparations used for inoculations in experiment 1 were kept in the refrigerator throughout the 12-day immunization period. By intracerebral inoculation of monkeys, active virus was demonstrated in the eluate at the beginning and at the end of the series of injections. A monkey was inoculated intracerebrally with virus-gel at the beginning of the experiment and died in 4 days with a marked drop in temperature and general apathy, without evidence of poliomyelitis. At the end of the 12-day period active virus was demonstrated in the eluate from virus-gel which had been stored. This indicates that potent virus was present in the immunizing preparations throughout the period of injections.

Experiment 2.—Six monkeys were given single subcutaneous injections of 10 cc. of virus-gel, representing 0.3 gm. of original tissue. Approximately 1 month later they were bled for serum and given virus intranasally. None survived the latter test and neutralizing serum was demonstrable in only one case. The results do not confirm the suggestion from experiment 1 that a

18. J. Exper. Med. 56: 307, 1932.

19. Proc. Soc. Exper. Biol. & Med. 30: 1010, 1933.

single injection of virus-gel is sufficient to induce the appearance of neutralizing antibody. The discrepancy may have been due to a difference in the virus-gel inoculums of the 2 experiments. Although both apparently were prepared in the same way, the inoculations in experiment 2 were followed by transient fevers and some local induration which was not seen in experiment 1. The poor immunological response was not due to absence of virus, however, since virus was demonstrated at the site of inoculation 4 days after the injection of one animal (experiment 7).

Experiment 3.—In order to detect any advantage of virus-gel as an immunizing agent, an experiment using crude virus was planned to parallel the virus-gel inoculations of experiment 1. Five cc. quantities of a 6% emulsion of crude virus were used for subcutaneous inoculations at 3-day intervals. This represents the amount of cord tissue used to make each 10 cc. quantity of virus-gel used in experiment 1. Of the first series of 6 monkeys, 3 died of intercurrent disease; although the remaining 3 failed to develop poliomyelitis following intranasal inoculation, this test was unsatisfactory inasmuch as the control died of intercurrent infection before showing poliomyelitis. The series was repeated and the 9 monkeys, excluding those dying of intercurrent infection, are grouped together in table 2. Two monkeys were attacked with poliomyelitis as a result of the immunizing injections. Two of the 3 in the first series, which failed to come down following intranasal inoculation, later resisted intracerebral inoculation. However, these 3 are not included in the final summary due to the unsatisfactory nature of the intranasal test (nonspecific death of control). Of the 6 serums tested in this series, 2 neutralized virus.

TABLE 2.—*Active Immunization With Varying Doses of Crude Virus*

Monkey No.	Immunization (5 cc. Quantities Sub- cutaneously at 3-day Intervals)	Result of Intranasal Virus Inoculation	Result of Serum Neutralization Test
21	1 injection	?	+
22	1 injection	?	0
23	1 injection	Poliomyelitis	**
24	1 injection***		
26	3 injections	?	0
27	3 injections	Poliomyelitis	+
28	3 injections***		
31	5 injections	Poliomyelitis	0
32	5 injections	Poliomyelitis	0

+ = neutralization; 0 = no neutralization.

* Result unsatisfactory; test monkeys survived but control died of intercurrent infection on third day. Monkeys 21 and 26 later resisted intracerebral inoculation.

** Test monkey died of intercurrent infection.

*** Died of poliomyelitis as a result of the immunizing injections.

Experiment 4.—As a further method of comparing the immunizing capacity of virus-gel and crude virus, a quantitative serum neutralization test was done using pooled serums of the 2 groups of monkeys. These serums were pooled in such a way that each mixture contained equal parts of serum from the 1-injection group of monkeys, from the 3-injection group, and from the 5-injection group. The serum mixtures were tested undiluted, and diluted 1:5 and 1:25. The results of this test are included in the summary given in table 5.

Since almost all of the immunized monkeys failed to resist intranasal test inoculation, the serum neutralization tests must serve for comparison of the various virus preparations as antigens. If the results of the virus-gel group (6 serums neutralized of 11 tested) are compared with the results of the control groups (8 serums neutralized of 12 tested in eluate and crude virus groups), there appears to be no greater immunological response when virus is combined with alumina gel. The response appears to be equal also in the two series, virus-gel and eluate, in experiment 1. In addition, the results of the one quantitative neutralization test show only a slight difference between the 2 serum mixtures and are essentially what one would expect if the positive serums of each group showed similar low neutralizing ability.

EXPERIMENTS WITH SHEEP

A number of attempts have been made to prepare poliomyelitis antiserum in large animals, since therapeutic value, measured by neutralizing titer, has been ascribed to such serums. Horses,²⁰ sheep,^{21a, b} and goats,^{21b} have been used with varying success. Virus inoculations have usually been continued over a long period of time, in some instances years, before potent neutralizing serums were demonstrated.

A sheep (L), undergoing a series of injections of crude virus for the purpose of producing an antiserum, was given two subcutaneous injections of virus-gel (50 cc. and 65 cc.) with a 3-week interval. A control sheep (R), receiving normal nervous tissue, was given 2 similar injections of nervous tissue-gel. Bleedings were made at intervals and neutralization tests done with representative serum samples. After it was evident that the control sheep (R) showed no consistent neutralizing ability, it also was injected with virus-gel. At the same time another sheep (N) was given virus-gel. The first results with these sheep were reported previously.^{2b} The complete record now appears in table 3.

Although the response of sheep L was good, one cannot evaluate accurately the efficacy of virus-gel injections in the presence of the previous crude virus injections. The highest neutralizing titer found after virus-gel immunizations was 1:500, while the serum showed a titer of at least 1:100 (end point not determined) before virus-gel injections were started. Both sheep R and N failed to produce a minimum serum titer of 1:25 following one injection of virus-gel. After a second virus-gel injection and one of crude virus, both had a titer of 1:10 and perhaps 1:100 in the case of R. Further evaluation of virus-gel as an immunizing agent in sheep was not possible, since this work was necessarily discontinued. In general, the response appears to have been speedier than that of previous reports in which unadsorbed virus was used. The repeated injections of both crude tissue and tissue-gel were well tolerated.

SURVIVAL OF ADSORBED VIRUS IN VIVO

Glenny, Buttle and Stevens²² and Hektoen and Welker⁵ have attributed the increased antigenicity of substances in combination with colloids to a slow release from the site of inoculation. The result is a continued stimulation to antibody production over some length of time following a single injection. In studying the antigenicity of virus-gel a determination of the survival of

20. Neustaedter, M., and Banzhaf, E. J.: *J. A. M. A.* **68**: 1531, 1917; Pettit, A.; *Bull. gén. de thérap.* **176**: 389, 1925; Weyer, E. R., Park, W. H., and Banzhaf, E. J.: *Am. J. Path.* **5**: 517, 1929; Morgan, W. T., and Fairbrother, R. W.: *Brit. J. Exper. Path.* **11**: 512, 1930; Howitt, B.: *Science* **80**: 621, 1934; Schaeffer, M.: *Proc. Soc. Exper. Biol. & Med.* **31**: 1232, 1934; Schultz, E. W., and Gebhardt, L. P.: *J. Immunol.* **26**: 93, 1934.

21. (a) Flexner, S., and Lewis, P. A.: *J. A. M. A.* **55**: 662, 1910; Krause, R.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **9**: 117, 1911; Pettit, A.: *Compt. rend. Soc. d. biol.* **81**: 1087, 1918; Stewart, F. W., and Haselbauer, P.: *J. Exper. Med.* **48**: 449, 1928; (b) Howitt, B.: *J. Infect. Dis.* **50**: 26, 1932; **53**: 145, 1933; Schultz, E. W., and Gebhardt, L. P.: *J. Immunol.* **26**: 93, 1934.

22. *J. Path. & Bact.* **34**: 267, 1931.

virus after adsorption should be of significance. This was found to be possible by examining the nodules which persist for many weeks following the subcutaneous inoculation of virus-gel. The nodules were dissected out and treated as described in the following experiments.

TABLE 3.—*Immunization of Sheep With Crude Virus and With Virus-Gel*

Date	History	Results of Serum Neutralization Test	
Sheep L			
November 3, 1933	Bled	Serum undiluted	0
November 7, 1933 to February 27, 1934	16 weekly injections of crude virus emulsion (50 cc. subcutaneously, 50 cc. intramuscularly)		
March 20, 1934	Bled	Serum undiluted	+
April 14, 1934	Bled	Serum diluted 1:25	+
		Serum diluted 1:100	+
	50 cc. virus-gel subcutaneously		
May 5, 1934	65 cc. virus-gel subcutaneously	Serum undiluted	+
June 2, 1934	Bled	Serum diluted 1:5	+
		Serum diluted 1:25	+
		Serum diluted 1:50	+
		Serum diluted 1:100	+
October 4, 1934	Bled	Serum diluted 1:25	+
		Serum diluted 1:50	+
		Serum diluted 1:100	+, +
		Serum diluted 1:250	+, +
		Serum diluted 1:500	+
		Serum diluted 1:1000	0
January 7, 1936	60 cc. crude virus subcutaneously		
January 28, 1936	Bled	Serum diluted 1:25	+
		Serum diluted 1:250	0
Sheep R			
November 3, 1933	Bled		0, 0, 0
November 7, 1933 to February 27, 1934	16 weekly injections of crude emulsion normal tissue (50 cc. subcutaneously, 50 cc. intramuscularly)		
March 20, 1934	Bled	Serum undiluted	0
April 14, 1934	50 cc. normal nervous tissue-gel subcutaneously		
May 5, 1934	65 cc. normal nervous tissue-gel subcutaneously	Serum undiluted	+, 0
June 2, 1934	Bled	Serum diluted 1:5	0, 0
		Serum diluted 1:25	0
October 4, 1934	Bled	Serum undiluted	+, 0
		Serum diluted 1:5	0, 0
		Serum diluted 1:25	+, 0
		Serum diluted 1:50	0
March 18, 1935	Bled	Serum undiluted	0
		Serum diluted 1:10	0
April 25, 1935	50 cc. virus-gel subcutaneously		
	Bled	Serum diluted 1:25	0
		Serum diluted 1:100	0
		Serum diluted 1:500	0
September 2, 1935	100 cc. virus-gel subcutaneously		
January 7, 1936	60 cc. crude virus subcutaneously		
January 28, 1936	Bled	Serum diluted 1:10	+
		Serum diluted 1:100	+, **
Sheep N			
March 18, 1935	Bled	Serum undiluted	0
March 20, 1935	25 cc. virus-gel subcutaneously		
April 25, 1935	25 cc. virus-gel subcutaneously	Serum diluted 1:25	0
	Bled	Serum diluted 1:100	0
		Serum diluted 1:500	0
September 2, 1935	100 cc. virus-gel subcutaneously		
January 7, 1936	60 cc. crude virus subcutaneously		
January 28, 1936	Bled	Serum diluted 1:10	+
		Serum diluted 1:100	0

+ = neutralization; 0 = no neutralization; each sign represents a separate test.

* These irregular results with sheep R are compatible with the observation that the serum of normal sheep at times may possess slight neutralizing ability (Stewart and Haselbauer^{21a}).

** The test monkey showed prolonged excitability and fever until sacrificed one month after inoculation. There was no histological evidence of poliomyelitis.

Experiment 5.—Thirty days after completion of the immunizing injections of experiment 1, the mass representing the final inoculation of monkey 6 was removed. In this case it was necessary to remove adjacent tissue and the emulsion, in 15 cc. of buffer solution at p_H 7.4, was shaken with ether in a manner similar to that used for purification of virus preparatory to ad-

sorption. The water layer after centrifugation was then concentrated by boiling in vacuo at 37 C., and injected intracerebrally into a monkey, which remained well.

Experiment 6.—Monkey 33 (6,000 gm.) was given 6 simultaneous injections of 5.0 cc. each of virus-gel at various points on the anterior body surface. One, 2, 4, and 8 days later, an injected mass was removed and emulsified in 10 cc. of buffer solution (p_H 7.4). After being shaken for one-half to one hour the emulsion was centrifuged and 1 cc. of the supernatant set aside. The remainder of the supernatant was concentrated by boiling in vacuo to a volume of approximately 1 cc. This concentrated material and the 1 cc. of supernatant that had been saved were then inoculated into opposite cerebral hemispheres of the same monkey. Both inoculations were made as it was not known whether such a process of concentration would destroy the virus. This series was to have been continued beyond the eighth day, but the animal supplying the virus-gel nodules came down with poliomyelitis on the tenth day. Poliomyelitis appeared in the monkeys which received material removed after 1, 2, and 8 days; the 4-day inoculation resulted in a nonspecific death.

TABLE 4.—*Survival in Vivo of Poliomyelitis Virus Adsorbed to Alumina-Gel*

	Virus-gel Injected Subcutaneously	Result in Injected Animal	Interval Before Removal of Injected Mass	Result of Intracerebral Injection of Eluate
Monkey 6 (exper. 5)	50 cc., 5 injections over 12 days	Immunization*	30 days	No poliomyelitis
Monkey 33 (exper. 6)	30 cc., 6 injections at one time	Poliomyelitis	1 day 2 days 4 days 8 days	Poliomyelitis Poliomyelitis Death on 4th day from cerebral trauma Poliomyelitis
Monkey 20a	10 cc., 1 injection	No immunity**	4 days	Poliomyelitis
Monkey 20b (exper. 7)	10 cc., 1 injection	No immunity***	16 days	Died of dysentery on 9th day; no poliomyelitis

* Serum neutralized.

** Failed to resist intranasal inoculation.

*** Serum did not neutralize; failed to resist intranasal inoculation.

Experiment 7.—Two monkeys were used in conjunction with experiment 2, each receiving a single subcutaneous injection of 10 cc. of virus-gel. One nodule was removed after 4 days and the other after 16 days. They were handled in the manner described in experiment 6 and injections of both concentrated and unconcentrated supernatant made into test monkeys. The 4-day material produced poliomyelitis. The monkey receiving 16-day material died of dysentery on the ninth day with no clinical or histological evidence of poliomyelitis.

In this series of experiments, summarized in table 5, active virus was demonstrated in material removed after 1, 2, 4 and 8 days, but not after 16* and 30 days, from the site of subcutaneous inoculation of virus-gel in monkeys.

TABLE 5.—*Summary of the Results of Active Immunization*

Immunizing Material	Number of Monkeys	Poliomyelitis from Immunizing Injections	Intranasal Inoculation		Serum Neutralization		Pooled Serum Neutralization
			Tested	Resisted	Tested	Neutralized	
Virus-gel	12	1	10	1	11		{ Undiluted + 1:5 0 1:25 0
Eluate	6	0	6	1	6	6	
Crude virus	12	2	4	0	6	2	{ Undiluted 0 1:5 0 1:25 0
Totals	30	3	20	2	23	14	

+ = neutralization; 0 = no neutralization.

* This test animal survived 9 days before dying of dysentery, a period slightly longer than the average incubation period but not as long as the maximum.

PASSIVE IMMUNIZATION

In the course of previous unpublished work²³ in this laboratory upon passive immunization against experimental poliomyelitis, one experiment suggested that massive transfusion of blood from convalescent monkeys might protect normal monkeys from inoculation with the virus. This experiment was performed as follows: After taking 70 cc. quantities of blood from 2 normal monkeys they were given 90 cc. of convalescent monkey blood intravenously and 95 cc. intraperitoneally. Both survived subsequent intracerebral inoculations of virus which caused poliomyelitis in a control. In the present work further investigation was done along this line by methods which followed closely those of the earlier experiment.

Experiment 8.—Blood taken from 6 convalescent monkeys by cardiac puncture was citrated and pooled. Normal monkey blood was collected in the same way. Four normal monkeys were bled from the heart for 50 cc. each and immediately each was given 70 cc. of the pooled monkey blood into a leg vein, 2 receiving convalescent and 2 normal blood. During the following 2 days these 4 monkeys and 2 untreated controls received 3 intranasal inoculations of virus. All developed poliomyelitis.

Two previous in vitro neutralization tests were done with pooled serum derived from the same monkeys, and in the same proportions, as furnished the pooled blood for the last experiment. Virus was neutralized by this serum when it was undiluted but not when diluted 1:5, 1:10, or 1:15. Since blood of a higher titer might give a different result in passive immunization, hyperimmunization of a group of monkeys was begun for use in the next experiment. Rhoads²⁴ has shown that this procedure increases the in vitro neutralizing titer of convalescent monkey serum. Four convalescent monkeys with residual paralysis and one monkey which had received a series of intraperitoneal and intradermal immunizing injections more than 2 years previously, were given intradermal injections of 10% virus in 2 cc. quantities at intervals of 3 or 4 days for one month. Pooled serum taken one month later and tested in vitro neutralized virus when undiluted, and diluted 1:5 and 1:15, the only dilutions tested.

Experiment 9.—Blood taken from the hyperimmunized monkeys was citrated and pooled in approximately the same ratio as that for the previous serum neutralization test. Four normal monkeys which had been bled by cardiac puncture for 20 cc. were inoculated intravenously as before, 2 receiving 60 cc. each of hyperimmune blood, and 2 receiving similar amounts of normal monkey blood. Again 2 untreated controls were included, making a total of 6 monkeys which received an intranasal instillation of virus on the second and third days after the transfusions. All developed poliomyelitis.

No evidence was obtained, therefore, that transfusion of immune blood can protect monkeys against direct (intranasal) inoculation of virus under these conditions. This is essentially in agreement with attempts to induce passive immunity by injections of serum. In this laboratory²³ a number of experiments were done in which immune serum (monkey and human) was given to monkeys by various routes. Evidence of protection by this means was quite irregular whether serum was given before or after the virus inoculation, and if the entire series is totalled we find that 64% of 31 test animals, and 66% of 22 controls developed poliomyelitis. Others have had similar experiences.²⁵

However, there is evidence that a quantitative factor may be significant.

23. Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B.; Unpublished work.

24. J. Exper. Med. **53**: 137, 1931.

25. For a review of experimental serum treatment see: Harmon, P. H.: Am. J. Dis. Child. **47**: 1179, 1934.

Schultz and Gebhardt²⁶ found that an antipoliomyelitis horse serum of high titer shows some evidence of protection when given previous to the virus, and prophylactic and therapeutic value has been attributed to a concentrated antipoliomyelitis horse serum.²⁷ In the previous experiment mentioned above which seemed to show protection of the monkeys, appreciably larger amounts of blood were given than in the two subsequent experiments. Shortage of convalescent monkeys prevented the exact repetition of this experiment.

COMMENT

Four monkeys developed poliomyelitis as a result of subcutaneous inoculation of virus: One had received four 10 cc. quantities of virus-gel* (exper. 1), two received 1 and three 5 cc. injections of crude virus respectively (exper. 3), and one received 30 cc. of virus-gel at one time (exper. 6). With the exception of experiment 6, other monkeys in the same series received the same and larger amounts of virus without showing infection. This indicates that the size of the subcutaneous dose was not the factor which determined whether infection was to occur. Also there is no evident correlation between the amount of virus given and the appearance of neutralizing antibody. Unknown factors determine the result of a subcutaneous inoculation of virus.

The appearance of poliomyelitis following subcutaneous injection of virus-gel indicates that these preparations were not entirely harmless. Since our purpose was primarily to enhance the antigenicity of virus we did not especially desire a noninfectious immunizing agent and consequently did not test our preparations for complete adsorption of virus. Similar procedures in other hands^{14,18,28} have given apparently complete adsorption. Although Rhoads¹⁴ found his virus-gel mixture innocuous when inoculated intracerebrally, Schaeffer and Brebner,²⁸ and Kramer, Grossman and Hoskwith¹⁸ produced poliomyelitis by intracerebral inoculation of such mixtures.

Since elution of virus from alumina gel is obtained in vitro by bringing the complex to a reaction of p_H 7.4, it is reasonable to suppose that a release of virus from the site of inoculation occurs, due to the influence of body fluids upon the hydrogen ion concentration of the inoculum. Any such release probably occurs slowly, as indicated by survival of virus at the site of inoculation for at least 8 days. However, no advantage of virus-gel as an antigen over eluate or crude virus was demonstrated by tests of resistance to direct inoculation or by neutralizing power of monkey and sheep serums. Since this work was concluded Olitsky and Cox¹¹ have come to similar conclusions in immunization experiments with equine encephalomyelitis virus adsorbed to alumina gel. They demonstrated virus at the site of inoculation 4 days after subcutaneous injection, but found no advantage of adsorbed virus over other preparations as an immunizing agent.

Table 5 shows that an immunological response to the various preparations

26. *J. Pediat.* **7**: 332, 1935.

27. Weyer, E. R., Park, W. H., and Banzhaf, E. J.: *J. Exper. Med.* **53**: 553, 1931.

* The fourth inoculation and possibly the third occurred within incubation period.

28. *Arch. Path.* **15**: 221, 1933.

of virus was detected in 14 of 23 monkeys whose serums were available for an *in vitro* neutralization test, while only 2 of 20 resisted intranasal inoculation of virus. Of 19 monkeys in which both tests were completed, 12 had neutralizing serum; among these were the 2 which resisted direct intranasal inoculation. This lack of correlation between the results of the serum neutralization test and direct inoculation of virus has been noted by all workers who have used both tests. An explanation of this discrepancy which has been suggested previously³ rests upon the present conception of the pathogenesis of the disease, the essential feature of which is the migration of virus in association with nerve fibers. With this as a basis it can be postulated that the resistance of a convalescent monkey to reinfection is due to previous contact of the central nervous system with virus, and the neutralizing antibody, under these circumstances, represents the response to virus in nervous tissue or to virus discharged from it. An antibody response is also evident when virus is inoculated extraneurally, but the contact between virus and central nervous system necessary to produce the disease or a resistance to it may not occur. The well-recognized blood-central nervous system barrier appears to be responsible for this lack of contact, since its disorganization by various means results in virus extraneurally inoculated producing the typical disease.²⁹ The failure of passive immunization may be explained upon the same basis. Although the animal may have large amounts of neutralizing serum introduced extraneurally, intranasal inoculation allows the virus to reach the central nervous system without being exposed to the action of an effective concentration of humoral antibody. Schultz and Gebhardt^{26,30} have expressed similar views regarding active and passive immunization.

Kramer, Grossman and Hoskwith¹⁵ injected virus-gel subcutaneously and report subsequent resistance to intracerebral inoculation in a majority of their monkeys. Intranasal inoculation was chosen by us because of the similarity to natural infection. The difference between routes of test inoculation in these two reports suggests itself as the reason for the discrepancy in results.

A disadvantage of intranasal inoculation may lie in the relatively large amount of virus used to insure infection; in many cases this may represent an overwhelming dose. When using the intracerebral route the dosage of virus can be carefully controlled.

SUMMARY

Subcutaneous inoculation of monkeys with crude virus or with virus-alumina gel preparations resulted occasionally in an attack of poliomyelitis.

Inoculation of the virus-alumina gel preparations subcutaneously into monkeys induced an immunological response, as shown by the development of neutralizing serum, to a degree at least equal to that of crude virus. No evidence was found that a better immunological response in monkeys could be obtained by this method.

Subcutaneous and intramuscular injections of crude virus and virus-

29. Lennette, E. H., and Hudson, N. P., *Proc. Soc. Exper. Biol. & Med.* **34**: 470, 1936.

30. *California and West. Med.* **43**: 111, 1935.

alumina gel preparations into sheep were followed by the appearance of neutralizing serum. The degree of antibody response of these sheep appeared to be greater than might be expected with crude virus alone.

Little or no evidence of resistance to intranasal test inoculation was found in monkeys which had received virus or virus and alumina gel subcutaneously.

Transfusions of blood from convalescent and hyperimmunized monkeys failed to induce immunity to intranasal inoculation in normal monkeys.

In these experiments neutralizing antibody, present in the blood as a result of either active or passive immunization, did not protect monkeys against intranasal inoculation of virus.

